THE STERNAL SCENT GLAND OF EURYCOTIS FLORIDANA (BLATTARIA: BLATTIDAE)

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THE STERNAL SCENT GLAND OF EURYCOTIS FLORIDANA (BLATTARIA: BLATTIDAE)[1]

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The cockroach *Eurycotis floridana* (Walker) secretes 2-hexenal in a large median ventral gland in the abdomen (Roth *et al.*, 1956). The gland is a lobed sac formed by an invagination of the epidermis between the sixth and seventh abdominal sternites. Two distinctly separated lobes of the gland arise on each side of the ventral nerve cord and extend forward to the third sternite (fig. 1). The large lobes consist of two or three less distinct lobes. When the gland is distended with fluid, its surface is smooth and the lobes are distinct. Tracheae, branching from the median ventral tracheal trunks, ramify extensively over the surface of the gland. Fat body is closely associated with the scent gland by means of tracheal attachments. Inconspicuous longitudinal muscles arise along the ventral surface of the two main lobes and insert near the median line at the anterior edge of the sixth sternite. No detectable nerves are connected with the gland. The gland opens to the exterior between the sixth and seventh sternites. A U-shaped indentation in the middle of the anterior margin of the seventh sternite delimits the dorsal side of the opening; the ventral side of the opening is slightly wider (fig. 2). Just posterior to the aperture of the gland is a crescent-shaped, rigid thickening in the intersegmental membrane (fig. 2,R). As there is no sphincter muscle at the opening of the gland and no muscles in the wall of the sac, the discharge of fluid from the gland seems to be controlled by the longitudinal muscles which attach the gland to the sixth sternite. Contraction of these muscles would elongate the sac and thereby decrease its volume and force the fluid out through the narrow slit of the closely opposed sternites (fig. 3). Similarly, general contraction of the abdomen would compress the fluid-filled sac and force out the liquid. The fluid can be ejected for a considerable distance (Roth *et al.*, 1956).

The scent gland is present and functional in adult males and females. If the cockroach is immobilized by cooling to 4°C, the sac can be dissected without having the fluid ejected. At emergence of the adult, no 2-hexenal could be seen in the scent gland but, when opened, the sac smelled faintly of the aldehyde. One day after emergence, small globules of secretion were visible; at four days, slightly larger globules had

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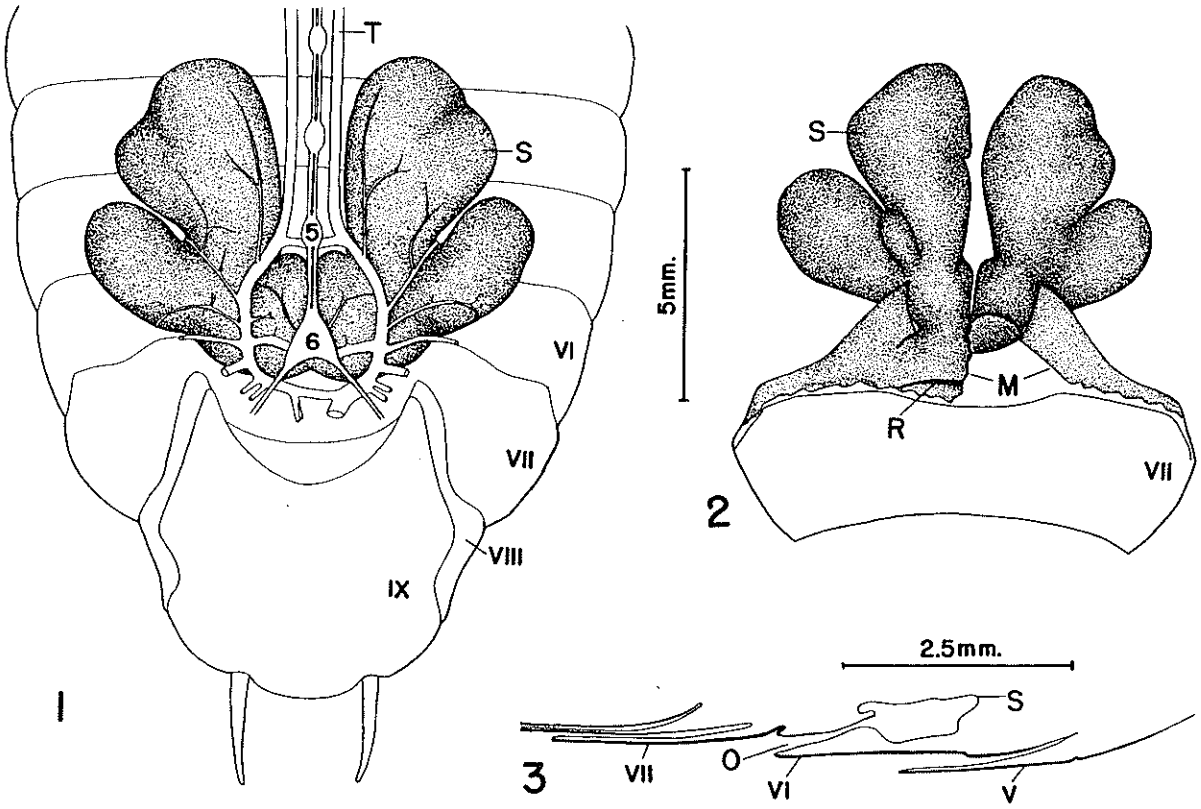
been formed. By nine days after emergence, the lobes of the gland were distended with large globules of secretion. In still older roaches, the gland was completely distended with secretion.

Scent glands were fixed in alcoholic Bouin's fluid, 80% alcohol, Helley's and Zenker's fluids. Sections, 5 μ thick, were stained with Delafield's hematoxylin, Mallory's triple stain, periodic acid Schiff (PAS) reagent (Lillie, 1948) and Gomori's (1952) alkaline phosphatase stain.

The wall of the sac is composed of an outer cuticular membrane, two cellular layers and an inner cuticular lining which is continous with the cuticle (fig. 4). Tall columnar secretory cells form the outer cellular layer, whereas the inner layer is formed of small squamous cells. The layer of secretory cells is discontinuous, so that between the clusters of secretory cells the wall of the sac is thin (fig. 4).

The secretory cell is characterized by a large, oval, basal nucleus, granular cytoplasm, and a cylindrical secretory apparatus. Within the striated secretory apparatus arises a cuticular tubule which extends up through the cell and along the surface of other cells (figs. 5, 6). In certain areas numerous tubules, sometimes as many as 25 or 30, open into the cuticular lining of the sac. Three tubules are shown opening into the lining in figure 10. Tracheoles penetrate between the secretory cells. The nuclei of the inner layer of cells are small but conspicuous (fig. 6); their cytoplasm is extremely thin and almost indistinguishable. Cell division was not observed in the adult gland.

The adult scent gland varied in its reaction to Schiff reagent after periodic acid oxidation. The purple staining material which was removed by pretreatment with saliva was considered to be glycogen. A series of scent glands from adults of various ages was studied in an attempt to coordinate the presence of glycogen with the secretory activity of the cells. Recently emerged adults were isolated and kept undisturbed from one to 33 days. Glycogen was more abundant in the cells of the scent glands of young cockroaches than in those of older insects. The cells of the scent glands of newly emerged cockroaches were laden with glycogen and all of the scent glands from the twelve cockroaches, less than two weeks of age, contained glycogen. On the



FIGS. 1–3. Semidiagramatic drawings of the scent gland of an adult male of *Eurycotis floridana*. 1. Dorsal view of the scent gland showing the position of the gland (S) in relation to the sternites (VI–IX), fifth and sixth ganglia (5, 6) and tracheal trunks (T). 2. Ventral view of scent gland (S) and seventh sternite (VII). The sixth sternite and part of the intersegmental membrane (M) have been cut away. Note the rigid area (R) in the intersegmental membrane. 3. Median longitudinal section through the scent gland (S) and sternites (V–VII) showing the opening (O) of the gland.

other hand, five of the twelve scent glands from cockroaches between 13 and 33 days of age lacked glycogen. A scent gland from a 27-day-old male *Eurycotis* was examined and found to lack any aldehyde odor. This scent gland was also devoid of glycogen. Four 13-day-old adults were made to eject their secretion by anesthetizing them with CO_2. Two days later, the gland sacs contained large globules of 2-hexenal but were not fully distended. The glands were fixed and stained for glycogen. The glands of four 15-day-old adults which had been isolated at emergence and kept undisturbed, were fixed as controls. Scent glands from three of the insects which had discharged the aldehyde two days previously contained considerable glycogen; one completely lacked glycogen. Of the controls, one showed no glycogen, one little glycogen, one a moderate amount, and only one considerable. The glycogen-filled secretory cells of a 7-day-old cockroach are shown in figure 8. Here a glycogen-laden cell is adjacent to one devoid of glycogen; this was a common occurrence. Also, in a single gland one may find groups of cells containing glycogen and groups without glycogen.

In addition to glycogen, other elements of the secretory cells were stained conspicuously with PAS. The tubules within the secretory apparatuses were stained red (figs. 6, 9), and the secretory apparatuses were frequently red but not as dark as the tubules. These elements were stained regardless of the fixatives used. On the other hand, distinct red granules surrounding the secretory apparatuses (fig. 9) were demonstrated after fixing in alcohol or Helley's but not after fixing in alcoholic Bouin's fluid. The presence of glycogen may obscure the granules but they were readily visible in preparations pretreated with saliva. The abundance of granules and glycogen was not always concurrent. Granules might be present in the absence of glycogen. The scent gland was unstained by Schiff reagent alone.

A most outstanding demonstration of the secretory apparatus was produced with Gomori's test for alkaline phosphatase. The area of the secretory apparatus was intensely blackened, whereas the nuclei and cytoplasm were lightly stained (fig. 7). The absence of glycogen in the secretory cells was not noticeably accompanied by a decrease in alkaline phosphatase.

In young nymphs the gland appears first as two tiny pointed sacs. In the last nymphal instar, the sacs are large, rounded and lobed. The muscles which attach the gland to the sixth sternite are more readily observed in the small nymphal gland than in the adult. At the end of the last instar, the gland is fully formed but apparently does not produce the odorous secretion.

Nymphal glands were stained with Mallory's triple stain, Feulgen reagent, PAS, and Gomori's alkaline phosphatase stain. The very small gland was composed of closely packed nuclei. Cell boundaries were indistinguishable. Mitotic figures appeared on the inner surface of the gland wall (fig. 11) and numerous chromatin bodies were discharged into the lumen. After the last nymphal instar had undergone about two-thirds of its development, mitosis ceased, the nuclei appeared in clusters, and cell boundaries were distinguishable in the apical cytoplasm (fig. 12). Figure 13 shows a distinct differentiation of cells in the nymphal gland. The apical cytoplasm of the clustered cells is dense and devoid of glycogen, and apparently pours out secretion. Nymphal glands were distended with viscous material which was preserved in fixation and was stained pink with PAS and blue with Mallory's triple

EXPLANATION OF FIGURES

FIGS. 4–13. Photographs of 5 μ cross-sections of scent glands of the cockroach *Eurycotis floridana* (figures 4 through 10, from adult males; figures 11 through 13, from nymphal males).

FIG. 4. Cross-section of one lobe of the scent gland. The columnar secretory cells occur in clusters, hence the thin areas in the wall of the sac. Note the cuticular lining. Alcoholic Bouin's, Mallory's triple. × 50.

FIG. 5. A portion of the gland cells shown in figure 4. The columnar secretory cells rest on a thin basement membrane and contain conspicuously stained secretory apparatuses. Above the secretory cells are dark nuclei of squamous epithelial cells and the membranous lining. × 880.

FIG. 6. A preparation fixed in alcoholic Bouin's solution stained with periodic acid-Schiff (PAS) reagent and haematoxylin. The secretory apparatus and the tubule within it are stained red but the cytoplasm is not colored. Note the nuclei of the squamous epithelial cells above. × 1590.

FIG. 7. Alkaline phosphatase is demonstrated in the region of the secretory apparatus with Gomori's procedure. × 1120.

FIG. 8. Glycogen is demonstrated with PAS reagent in secretory cells of a scent gland fixed in 80% alcohol. × 1120.

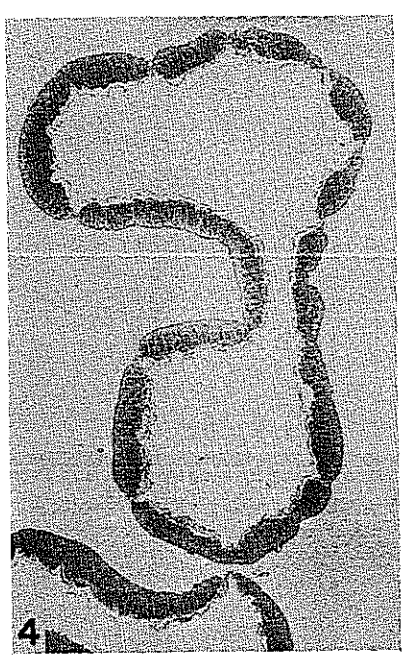
FIG. 9. Secretory apparatuses of the same gland pictured in figure 8. This section was pretreated with saliva and stained with PAS. The masses of glycogen are removed but red granules persist around the secretory apparatuses. Note in figure 6 that no granules are demonstrated with PAS after fixation in alcoholic Bouin's fluid. × 1120.

FIG. 10. Tubules which arise in the secretory apparatuses are shown opening into the cuticular lining. Alcoholic Bouin's, Mallory's triple. × 1880.

FIG. 11. Part of a cross-section of a small nymphal scent gland. Note the numerous small nuclei and mitoses adjacent to the lumen of the sac. Alcoholic Bouin's, Feulgen and light green. × 440.

FIG. 12. Part of a cross-section of a scent gland from an older nymph. No mitosis is visible; differentiation of cells is evident. Alcoholic Bouin's, Feulgen and light green. × 440.

FIG. 13. Nymphal gland similar to that of figure 12. Glycogen is demonstrated around the large nuclei. The clusters of cells with small nuclei are devoid of glycogen but are stained pink after pretreatment with with saliva. Alcoholic Bouin's, PAS. × 1120.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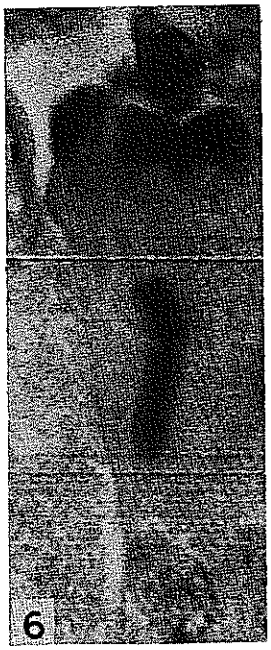
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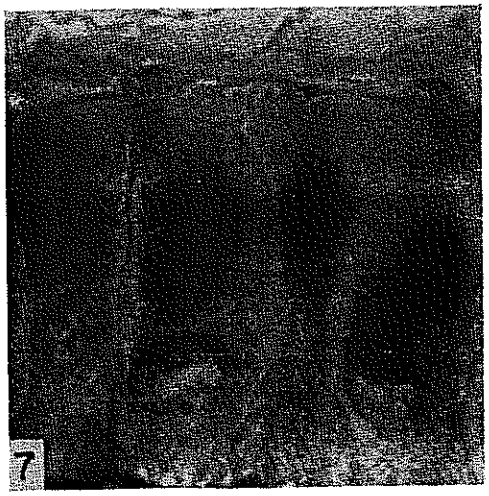
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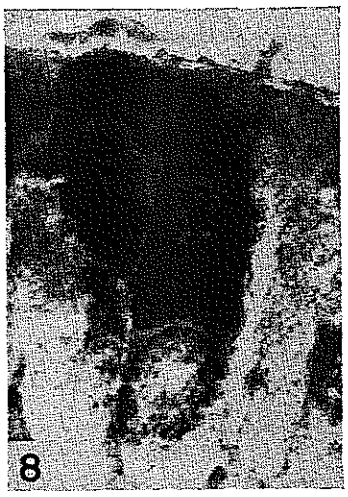
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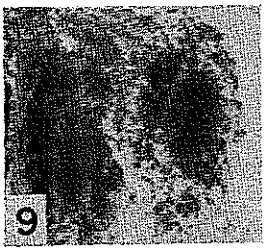
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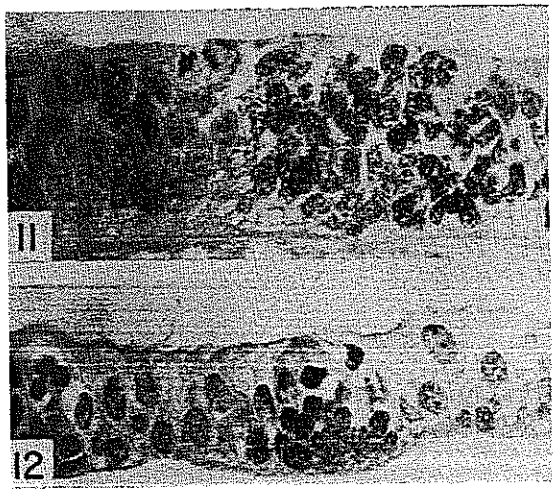
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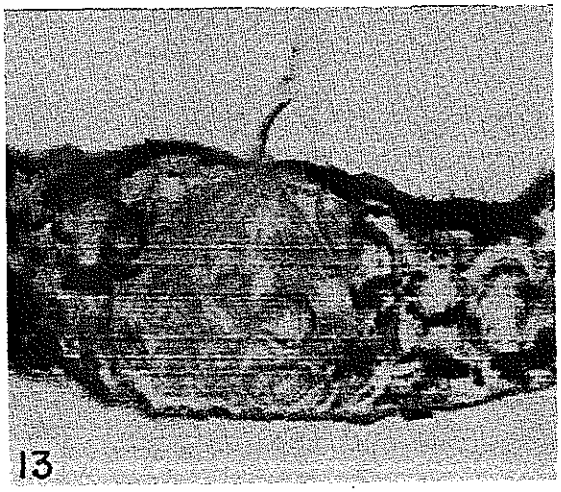
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stain. The cells between these clusters had larger nuclei (figs. 12, 13) and contained deposits of glycogen (fig. 13). During the last third of the final nymphal instar the cells continued to differentiate until, a few days before emergence, the gland appeared much like the double layered gland of the adult. Tall columnar secretory cells formed the outer layer of the gland. The inner layer was composed of small epithelial cells underlying a much-folded cuticular lining. Both cell layers were laden with glycogen. The tubules in the apexes of the secretory cells were stained pink with PAS reagent and, in some nymphs, were surrounded by granules which resisted treatment with saliva. Alkaline phosphatase activity in the region of the secretory apparatus was found only in the differentiated glands of the fully grown nymphs.

DISCUSSION

The morphology of the scent gland of *Eurycotis floridana* is similar to that of the sternal organ of *Blatta orientalis* L. (Harrison, 1906; Stanislavskij, 1926) and the ventral gland of *Periplaneta americana* (L.) (Liang, 1956). Similar glands have also been observed in *Neostylopyga rhombifolia* (Stoll) and *Platyzosteria novaeseelandiae* Brunner (Roth and Willis, unpublished). In size and shape, the gland of *Eurycotis* most closely resembles those of *Neostylopyga* and *Platyzosteria*. The gland of the oriental cockroach is less lobed and much smaller in proportion to the size of the insect.

The cellular structure of the gland of *Eurycotis* appears to be similar to that of *B. orientalis*. However, Harrison did not observe the continuity of the intracellular tubules of the gland cells with the tubules which join the lining membrane. She considered the inner layer of irregular nuclei to be degenerating gland cells which had migrated inwards. It seems unlikely that the gland cells degenerate, for no regenerative cells or mitotic figures have been observed in the adult gland cells, and the secretory cells do not appear in various stages of degeneration. It seems more likely that the inner layer of nuclei are comparable to the "chitinogenic" layer of cells (Konček, 1924) or "supporting cells" (Oettinger, 1906) described in the tergal glands of *Blattella germanica*. Konček believed these small cells were responsible for the formation of the secretory ducts as well as the chitinous covering. Stanislavskij (1926) recognized the similarity in structure between the sternal scent gland and other hypodermal glands of the oriental cockroach.

Although Roth (1952) failed to observe tubules in the cells of the tergal gland of males of *Supella supellectilium* (Serv.) a reexamination of his original slides shows that the cells are similar to those of tergal glands of males of other species of cockroaches. The secretory cells possess secretory apparatuses and tubules, and lie under a layer of small "chitinogenic" cells. The colleterial glands of the female *Periplaneta americana* (Linn.) (Brunet, 1951) and the male accessory glands of *Leucophaea maderae* (Fab.) (van Wyk, 1952) are examples of glands which have structures basically similar to the ventral scent gland and the various tergal glands already described.

There was no change in the size of the secretory cells or in the condition of their cytoplasm or nuclei which would indicate a drastic change in the physiological activity of the secretory cells. Only changes in the occurrence of glycogen and PAS-positive granules suggest a cyclic activity. From the brief survey of the occurrence of glycogen in the secretory cells, it is not possible to establish definitely a secretory pattern of the scent gland. However, if the presence of glycogen is an indication of secretory activity of the cells, it would appear that the scent gland pours out secretion until the sac is fully distended, tapers off secretion until the fluid is discharged, and then actively secretes again. The secretory activity of the cells is probably not synchronous, inasmuch as glycogen may be absent and present in adjacent cells.

The manner in which glycogen may be linked to the production of 2-hexenal is a matter for further biochemical investigation. The granules around the secretory apparatuses and the material within the secretory tubules, demonstrated by Schiff's reagent after periodic acid oxidation, are probably more closely related to the aldehyde. The secretory granules and the material within the tubules apparently differ in composition, for the tubular material stains after fixation in either alcoholic Bouin's fluid or alcohol, while the granules are not preserved with alcoholic Bouin's fluid.

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SUMMARY

1. The morphology of the sternal scent gland which secretes 2-hexenal in *Eurycotis floridana* is described.

2. Histologically, the gland consists of an outer layer of tall, columnar, epithelial cells, an inner layer of small, squamous, epithelial cells, and a cuticular lining. The secretory cells are characterized by secretory apparatuses and minute tubules which convey the secretion to the lumen of the sac.

3. Histochemical tests revealed the presence of glycogen and other periodic acid Schiff-positive material in the secretory cells and a high level of alkaline phosphatase activity in the regions of the secretory apparatuses.

4. The nymphal scent gland, though well developed in the last instar, does not secrete 2-hexenal. The development of the nymphal gland is described briefly.

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